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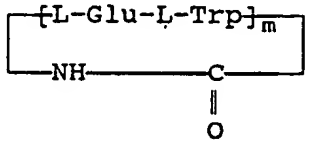

(I)

(57) Abstract

Methods are provided for the therapy of immunodeficient, immunodepressed or hyperactive immune states and for the prevention and treatment of opportunistic infections in such states comprising administering to a subject a pharmaceutically acceptable composition comprising as an active ingredient the dipeptide L-Glu-L-Trp, the cyclic monomer thereof, polymers thereof of the formula H_2N -{L-Glu-L-Trp}$_n$ - CO_2H; cyclic polymers thereof of formula (I) and their pharmaceutically acceptable salts thereof, wherein n and m are independently ≥ 2.

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PHARMACEUTICAL DIPEPTIDE COMPOSITIONS AND METHODS OF USE THEREOF

The present invention is directed to dipeptide pharmaceutical compositions and uses thereof, in particular, uses thereof for treatment of immunodepressed states and of opportunistic infections in immunodepressed states.

BACKGROUND OF THE INVENTION

Several polypeptides found in the thymus gland have been implicated as playing roles in the development and maintenance of immunological competence in animals, including human beings. Some of these polypeptides have been shown to stimulate the maturation, differentiation and function of T-cells. For example, a heat-stable fraction isolated from calf thymus extracts, designated as Thymosin fraction 5, has been shown to reconstitute immune functions in thymic-deprived or immunodepressed individuals. Several peptides have been isolated from Thymosin fraction 5, such as Thymosin $alpha_1$ (28 amino acids, U.S. Patent No. 4,079,127), Thymosin $beta_4$ (44 amino acids, Low et al., PNAS, 78,1162-1166 (1981)), Thymosin $beta_8$ (39 amino acids, U.S. Patent No. 4,389,343) and Thymosin $beta_9$ (41 amino acids, U.S. Patent No. 4,389,343). However, practical administration of such polypeptides is expensive due

to the relatively low yield and complexity of isolation and/or manufacture of such long chain polypeptides. Most importantly, in some cases, these polypeptides produce side reactions in patients.

The present invention is based in part on the discovery that a dipeptide, hereinafter referred to as Thymogen, exhibits a broad range of efficacy for prevention and treatment of opportunistic infections in immunodepressed states, and for therapeutically effective treatment of immunodeficient states. This is believed to be highly unexpected for such a relatively small compound to exhibit such a broad range of activity. Furthermore, we have not found any significant side effects from the use of the dipeptide according to the present invention. Due to its simple nature, the dipeptide is rather inexpensive to manufacture.

As used herein, the terms "immunomodulator" and "immunomodulating" encompass the activity of enhancing or restoring the subject's immune system, as evidenced by measurable blood parameters and/or the patient's improved ability to combat infection or disease, and the ability to heal tissue. Hence, immunomodulation encompasses improvement of the immune system due to an immunodeficient state (for example, caused by removal of the thymus), and/or an immunodepressed state (for example, caused by exposure to radiation). Furthermore, the present invention provides for modulation of the immune system by lowering blood parameters and other indicia of the immune state if these indicia are abnormally elevated. The present invention encompasses the therapeutic method of treating the immunodeficient, immunodepressed or elevated immune state <u>per se</u>, thus providing prophylaxis against infection and disease,

as well as a treatment of infection, disease or wound indirectly by enhancing the immune system.

It is therefore an object of the present invention to provide pharmaceutical compositions of the dipeptide Thymogen which have broad immunomodulating activity, as well as activity for other uses such as treatment of infections, disease and wounds (burns, frost bites, and the like), enhancement of metabolic processes, and many other uses.

It is an object of the present invention to provide therapeutic methods for treatment of immunodepressed and immunodeficient states.

It is yet another object of the present invention to provide methods for preventing and treating opportunistic infections in immunodeficient and immunodepressed states.

These and other objects will be apparent from the following description and appended claims.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides pharmaceutical preparations comprising the dipeptide L-Glu-L-Trp, including cyclic forms and linear and cyclic polymers of the dipeptide, using the normal convention wherein the first named amino acid is the amino terminus and the last named amino acid is the carboxyl terminus. For convenience, all forms will be collectively referred to herein as "the dipeptide". The compositions containing the dipeptide according to the present invention may be formulated into any convenient formulation which allows for the active ingredient to be absorbed into the blood stream.

Intramuscular and intranasal forms of application are preferred. The preferred dosage rate of the active ingredient for intramuscular administration is about 50 to 100μg per dose for adults (for a 300 to 1000μg total treatment therapy); for infants up to 1 year old about 10μg per dose, for infants 1 to 3 years old about 10 to 20μg per dose; for infants 4 to 6 years old about 20 to 30μg per dose, for children 7 to 14 years old about 50μg per dose. All of the foregoing dosages are useful for a treatment of 3 to 10 days, depending upon the immunodeficiency level. The treatment may be repeated as needed, usually within 1 to 6 months.

For prophylactic uses against opportunistic infections in immunodeficient or immunodepressed patients, the intramuscular and/or intranasal single daily dose for adults may be from about 50 to 10μg, and for children about 10 to 50 μg per dose for treatment over 3 to 5 days.

For treatment of burns, frost bite, or other wounds, including chronic apical periodontitis, the dipeptide may be applied in about 100μg doses as a paste or other suitable medium.

For ophthalmology, such as for treatment of infectious eye diseases, the dipeptide may be applied in single daily dosages of about 10μg (over 4 to 10 days) or as installations into the conjunctival cavity at about 5μg twice daily over about 4 to 5 days.

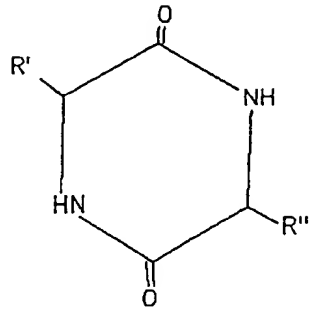
The dipeptide may be utilized intramuscularly as an injection solution with the active ingredient in a therapeutically effective immunopotentiating amount of about .001 to .01% by weight. If presented in the

form of a tablet, capsule or suppository it is preferred that the active ingredient be present in an amount of about 0.1mg per tablet, suppository or capsule. If presented in such form, the capsule, suppository or tablet may also contain other conventional excipients and vehicles such as fillers, starch, glucose, etc.

The dipeptide may be obtained by conventional peptide synthesis, including the Merrifield solid state peptide synthesis technique. Typically an amino and side chain protected derivative of an activated ester of glutamic acid is reacted with protected L-tryptophan. After elimination of the protecting groups and conventional purification, such as by thin layer or GL chromatography, the peptide may be purified such as by, lyophilization, gel purification, and the like.

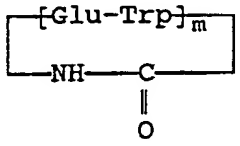
The purified dipeptide L-Glu-L-Trp, comprises a white powder (if lyophilized; otherwise, it is crystalline), soluble in water, DMF; insoluble in chloroform and ether. [alpha$^{22}_{D}$ = +12.6; C = 0.5 H_2O. R_f = 0.65 (butanol: acetic acid: water = 3:1:1). UV (275 $\pm$ 5nm, max). NMR (500MHz): 0.001mol/l of the peptide solution, Trp (3.17; 3.37; 4.57; 7.16; 7.24; 7.71; 7.49); Glu (1.90; 1.96; 2.21; 3.72).

Other forms of the dipeptide are also encompassed by the invention, including the cyclized monomer.



Wherein R′ and R″ are, respectively , the alpha-side chains of Glu and Trp;

Linear polymers H_2N —{Glu-Trp}$_n$— CO_2H, n≥2

and cyclic polymers  ; m≥2

all of these forms, including the dipeptide monomer, will be referred to as the "dipeptide". The linear polymers may be made by conventional peptide systhesis, including Merrifield solid-state peptide methodology. The cyclic monomer and polymers may be prepared by cyclizing the linear peptide in with peptide linkage agents in dilute solutions.

The active dipeptide ingredient of the pharmaceutical preparations according to the present invention may be used as a free peptide or in the form of a water soluble pharmaceutically acceptable salt, such as a sodium, potassium, ammonium or zinc salt. It will be understood that the dipeptide may be administered with other active ingredients which independently impart an activity to the composition, such as, antibiotics, interferon, anesthetics, and the like.

The most preferred formulation according to the present invention is a solution for intramuscular injection containing about .001 to .01% by weight (.0001-.001mg/kg body weight, or 10-100μg active ingredient per 1ml solvent). The pharmaceutically acceptable vehicle for this injection form may be any pharmaceutically acceptable solvent such as 0.9% aqueous sodium chloride, distilled water, Novocaine solution, Ringer's solution, glucose solution, and the like. The dipeptide containing compositions according to the present invention may be administered in a compatible pharmaceutical suitable for parenteral administration (e.g., intravenous, subcutaneous, intramuscular). The preparations may be subjected to conventional pharmaceutical operations, such as sterilization, and may contain adjuvants, such as preservatives, stabilizers, wetting agents and the like.

The pharmaceutical preparations according to the present invention demonstrate a high effectiveness in the treatment of immunodepressed and immunodeficient states for the preventing and treatment of opportunistic infections in those states.

Also included within the scope of the present invention are the pharmaceutically acceptable salts of the dipeptide, such as sodium or potassium or strong organic bases, such as guanidine.

The dipeptide containing compositions according to the present invention have activity in the restoration and stimulation of the immune functions. Thus they are useful in the treatment of opportunistic infections of an immunodepressed subject in an immunopotentiating effective amount as described above.

The dipeptide compositions according to the present invention may also be used in veterinary practice as an immunomodulatory agent for prophylaxsis and treatment of hypotrophy in farming animals, fur bearing animals and poultry.

Among the opportunistic infections which may be treated utilizing the compositions according to the present invention are: respiratory diseases, influenza, AIDS, burns, wounds, other open sores, rashes (due to allergic reactions), sun exposure, local trauma (with an ointment), eczemas, psoriasis, and the like. Furthermore, the compositions according to the present invention may be utilized to assist healing in immunodepressed or immunodeficient states, such as for the healing of bone fractures, lesions, gingival diseases, gynecological infections, infralymphatic infections, and the like. The compositions may also be used to enhance the immunodeficient state to increase susceptibility to microbial antibiotics and to enhance the patient's responsive reaction to other types of therapies.

The compositions according to the present invention also may be utilized to enhance metabolic processes; to enhance production of blood insulin; for treatment of irradiated cancer patients, as well as for veterinary uses.

Another important use is the treatment of chronic fatigue syndrome (CFS), which is believed to be a manifestation of an immunodepressed state.

The following examples are provided to further elucidate the invention, but are not intended to restrict the invention in scope or spirit in any way.